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# BACTERIA IN COMMERCIAL BOTTLED WATERS.

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## INTRODUCTION.

During the last six years from 1 to 17 samples of bottled waters from each of 110 American springs and from 57 sources in foreign countries have been examined in the Bacteriological Laboratory of the Bureau of Chemistry.[1] A comparative study of the results obtained should, therefore, contribute toward the formation of an opinion as to the freedom from contamination which we have a right to expect and to demand in the case of this product. These bacteriological analyses have been brought together and tabulated; and the results of this study have been considered to determine whether the standard adopted by the United States Public Health Service[2] for water on trains could be fairly applied to bottled waters, or whether some other standard would be more just.

A questionnaire was also sent out to a number of bacteriologists who have been associated with sanitary and allied problems. This questionnaire was arranged primarily to learn the attitude of a widely distributed group of workers in regard to bacterial tolerance in bottled waters. Of the 49 correspondents who have replied, 8 had not worked upon water sufficiently to feel competent to express any opinion. The remaining 41 replies are summarized as follows: Eight (19.8 per cent) stated that to them the term "bottled water" implied an unwritten guaranty of absolute purity;" five (12.1 per

[1] Examinations were made by various members of the Bacteriological Laboratory, including Dr. Geo. W. Stiles, Minnie Jenkins, Carleton Bates, Ruth C. Greathouse, and the author.

The author wishes to acknowledge the valuable assistance rendered by Dr. Charles Thom in the preparation of this paper.

[2] U. S. Public Health Reports, 1914, p. 2959. (Not more than one out of five 10 cc portions shall show gas.)

cent) desired no rigid standard; only one desired a standard of no *B. coli* in 10 cc quantities; thirty-five (85.4 per cent) desired to apply the Hygienic Laboratory standard or one more rigid; eight (19.8 per cent) would tolerate no *B. coli* in bottled waters; one of the five bacteriologists desiring no rigid standard considered water to be suspicious if three 10 cc portions show *B. coli*.

We have a right to demand that bottled water shall first of all be clean. Whatever other qualities it may claim or offer are secondary to cleanliness. In a study, therefore, of the bacteria found, we have a right to consider them not only as possible evidences of danger to health but as indices of conditions in the bottling room for which the operator is clearly responsible.

## SIGNIFICANCE OF BACTERIA IN POTABLE WATERS.

It is understood that natural waters may contain bacteria which multiply in the presence of very small amounts of organic matter. Bacteriologists who have worked with distilled water are familiar with the micrococci which multiply rapidly therein when the percentage of organic material is extremely low. The presence, therefore, of a large number of organisms in waters which have been bottled for several days or weeks has little significance unless the characters of these organisms are more or less definitely known.

The presence of *B. coli* in large numbers in waters is universally considered as an indication of the possible presence of its dangerous associates. The conditions under which waters are bottled and held and the mineral substances present may, in some cases, exert influences upon the multiplication of *B. coli* differing slightly from the effect of surface or well waters in nature. Preliminary studies in this laboratory indicate an immediate decrease instead of any possible increase of *B. coli* in freshly inoculated bottles of certain spring waters.[1] Houston[2] found that *B. coli* disappeared in stored water from the River Lea. Dunham[3] observed that distilled water enriched with either hay infusion or nutrient broth (1 cc in 1 liter) and inoculated with over 20,000 *B. coli* showed a marked reduction of the total number of *B. coli* at the end of 24 hours. He also reported that sterile water inoculated with pollution from ordinary soil does not show an appreciable number of *B. coli*.

It may, therefore, be assumed that bottled waters in which *B. coli* are found in appreciable numbers contained approximately all of those *B. coli* (if not more) when they left the springs or bottling

[1] Browne, W. W. (Jour. Infect. Dis., v. 17, No. 1, 1915, pp. 72–78) finds multiplication of *B. coli* in stored water, but an analysis of his experiments shows that the water used was so enriched as to be no longer comparable to stored spring waters.

[2] Houston, Reports on Research Work, Metropolitan Water Board, London, 1907.

[3] Dunham, E. K., Value of bacteriological examination of water from a sanitary point of view, Jour. Amer. Chem. Soc., v. 19, No. 8, 1897, p. 591.

houses. It is reasonable also to assume that when people pay from 2 cents to $30 per gallon for bottled water they expect to obtain a pure, or at least a safe water. Whipple[1] has defined a "pure" water as one which is "free from bacteria or other organisms which are liable to cause disease, and also free from *B. coli*."

## INSPECTION OF SPRINGS.

The ultimate test of the fitness of a particular water for sale lies in its condition at the spring. When contaminations are found in the bottled article, the determination of responsibility for the condition found calls for inspection at every stage of its handling. Such inspections of springs have been made from time to time, usually resulting in locating the source of trouble. The results of the inspection of three springs are included in Tables I, II, and III. These illustrate certain typical sources of pollution. In spring No. 1, insufficient coverings over the spring evidently permitted the entrance of a rotten lemon or orange, containing the mold *Penicillium italicum*, a short time previous to the collection of these samples. This mold can not exist long in water, and is practically never found except on decaying citrus fruits. The actual inspection of this spring and statements by the people of the vicinity disclosed the fact that freshets would cause the water in the creek flowing past to back through a swimming pool and into the spring. Inadequate care was also apparent in the method of cleaning and rinsing the bottles before they were filled. These bottles, as were those used at spring No. 3, were rinsed with polluted water just before filling. (See Table III.) The water in spring No. 2 was undoubtedly grossly polluted at times from the creek which flowed past. A culture of *B. paratyphosus B* was obtained from a shipment of bottled water from this spring four months prior to the inspection.

It is not always possible, however, to locate the source of contamination at the spring even by several inspections. One such spring is still under observation. This spring is on high land well removed from farm buildings and large streams of surface water. Its water is highly mineralized and at its source contains *B. coli* in 1 cc or 0.1 cc quantities. It is said that the water is boiled and the bottles sterilized before the bottling; yet 88 out of 96 bottles purchased at retail stores have been found to contain *B. coli* in 10 cc quantities, and 64 out of 96 in 1 cc quantities. The *B. coli* found were identified in all instances as belonging to the *communis* and *communior* groups. Evidently the survey has been incomplete in some essential point.

Naturally carbonated waters occasionally contain large numbers of organisms. In general, however, artificially carbonated waters

[1] Whipple, Geo. C., Value of pure and wholesome water, Biol. studies of the pupils of W. T. Sedgwick, June, 1906.

were found to contain no *B. coli* in 10 cc quantities and very low total counts at both temperatures of incubation. The total counts very seldom were above 50 per cc, and often were less than 10 per cc.

In certain instances legal actions have been brought against companies preparing and selling bottled waters when the waters examined have contained an excessive number of organisms, including *B. coli*. These companies having been thus impressed with the necessity of producing a clean commercial product have responded by placing on the market later consignments from which no *B. coli* were isolated in 10 cc quantities from 12 or more bottles. Repeated examinations of water from many springs have failed to show any *B. coli* in 10 cc quantities.

## EXAMINATION OF COMMERCIAL BOTTLED WATERS.

The methods employed in making these bacterial examinations were those prescribed from year to year by the committee on water analysis of the American Public Health Association. The high-temperature counts have always been made on plain agar after incubation at 37° C.; but the earlier low-temperature incubations were made on agar at 25° C., instead of on gelatin at 20° C., as during the last two years. Dextrose broth, lactose bile, and lactose broth have been used at different times for the preliminary tests for *B. coli*; but in nearly every instance, when reported present, *B. coli* have been isolated. Many of these have been verified by testing special dextrose cultures with methyl red, as recommended by Clark and Lubs.[1] A summary of all these examinations follows:

Of 110 domestic springs (see Table IV)—

47 (43 per cent) contained no *B. coli* in 10 cc quantities.
63 (57 per cent) contained *B. coli* in 10 cc quantities.
61 (55 per cent) contained *B. coli* in 5 cc quantities.
59 (53 per cent) contained *B. coli* in 1 cc quantities.[2]
49 (44 per cent) contained *B. coli* in 0.1 cc quantities.
31 (28 per cent) contained *B. coli* in 0.01 cc quantities.
10 (9 per cent) contained *B. coli* in 0.001 cc quantities.[3]

Sixty-nine (62 per cent) gave counts of less than 100 per cc on one or more bottles after incubation at 37° C. for two days.

Eighteen (16 per cent) gave average counts of less than 100 per cc on six or more bottles at 37° C.

Fourteen (12 per cent) gave no counts of less than 1,000 per cc on six or more individual bottles.

The highest average count on all samples from any one spring was 191,238.

[1] Clark and Lubs, The differentiation of bacteria of the Colon-aerogenes family by the use of indicators Jour. Infect. Dis., v. 17, No. 1, 1915, p. 160.

[2] Any potable water supply containing *B. coli* in 1 cc quantities is considered suspicious by health departments and is at once investigated.

[3] Water containing *B. coli* in 0.001 cc quantities is too suggestive of dilute sewage to be accepted by anyone.

Of 57 foreign springs (see Table V)—

29 (51 per cent) contained no *B. coli* in 10 cc quantities.
28 (49 per cent) contained *B. coli* in 10 cc quantities.
25 (45 per cent) contained *B. coli* in 5 cc quantities.
21 (37 per cent) contained *B. coli* in 1 cc quantities.[1]
16 (28 per cent) contained *B. coli* in 0.1 cc quantities.
8 (14 per cent) contained *B. coli* in 0.01 cc quantities.
2 (3 per cent) contained *B. coli* in 0.001 cc quantities.[2]

Forty (70 per cent) gave counts of less than 100 on one or more bottles after incubation for two days at 37° C.

Twenty-five (44 per cent) gave average counts of less than 100 per cc at 37° C.

The highest count shown at 37° C. was 37,000 per cc. This sample gave an average count of 16,000 per cc, and *B. coli* were found in one-third of the bottles examined in 5 cc quantities.

Two imported waters bearing on their labels the words "bacteriologically pure" gave the following results:

Sample No. 1; six bottles examined—

| | |
|---|---|
| Lowest number of organisms per cc developing on gelatin at 20° C..... | 700 |
| Average number of organisms per cc developing on gelatin at 20° C..... | 2,450 |
| Lowest number or organisms per cc developing on agar at 37° C..... | 300 |
| Average number of organisms per cc developing on agar at 37° C..... | 1,250 |

4 bottles contained *B. coli* in 10 cc quantities.
4 bottles contained *B. coli* in 5 cc quantities.
4 bottles contained *B. coli* in 1 cc quantities.
2 bottles contained *B. coli* in 0.1 cc quantities.

Sample No. 2; seven bottles examined—

| | |
|---|---|
| Lowest number of organisms per cc developing on gelatin at 20° C..... | 120 |
| Average number of organisms per cc developing on gelatin at 20° C..... | 9,410 |
| Lowest number of organisms per cc developing on agar at 37° C..... | 40 |
| Average number of organisms per cc developing on agar at 37° C..... | 482 |

6 bottles contained *B. coli* in 10 cc quantities.
5 bottles contained *B. coli* in 5 cc. quantities.
5 bottles contained *B. coli* in 1 cc quantities.
5 bottles contained *B. coli* in 0.1 cc quantities.
3 bottles contained *B. coli* in 0.01 cc quantities.

Among the organisms which have been isolated from the above samples are: *B. coli*, *B. cloacæ*, *B. mycoides*, *B. paratyphosus B*, *B. aerogenes*, *B. aurantiacus*, *M. citreus*, *B. maritimum*, *B. ovale*, *B. prodigiosus*, *B. fluorescens liquefaciens*, *B. fluorescens non-liquefaciens*, *B. subtilis*, and long-chain streptococci.

Molds of the genera Trichoderma, Penicillium, Cladosporium, Citromyces, Fusarium, Actinomyces, and Sporotrichum were identi-

[1] Any potable water supply containing *B. coli* in 1 cc quantities is considered suspicious by health departments and is at once investigated.

[2] Water containing *B. coli* in 0.001 cc quantities is too suggestive of dilute sewage to be accepted by anyone.

fied.[1] Without attaching too much significance to the occurrence of any of these forms, it may be remarked that Actinomyces and Sporotrichum are both large ill-defined groups, some of whose members are pathogenic to man as well as to other animals. A large number of spores of a species of Actinomyces culturally resembling the pathogenic form were found in one imported water. Similarly, Sporotrichum in large numbers was found in another water as taken in the market and as taken directly from the spring three months later. While proving nothing, such observations do not add to the attractiveness of such waters. The other genera listed are regularly found in soil and in decaying vegetable matter. Sufficient to say, they are not indicative of cleanliness.

## CONCLUSIONS.

Bottled water for table use should either be actually sterile or should comply with a strict standard as to the number of *B. coli* tolerated.

No water should be permitted to be sold which is contaminated at the source in any manner.

Inspection of springs and bottling establishments together with the analysis of official samples indicates that ignorance of proper precautions, carelessness, and neglect, are fully as large factors in the contaminations found as are impurities actually present in the springs.

The numbers of *B. coli* in official samples collected in the market may be safely assumed to be less rather than greater than the numbers in the freshly bottled stock.

The data as summarized show the need of improvement in the bacteriological condition of many of the brands of bottled water to be found in the market. Careful consideration of cases to which special study has been given shows that there are some springs used for the production of commercial bottled waters which should not be so used. It is evident that the presence of serious and unremovable contamination should shut the water of a spring permanently from the market. Such contamination could easily be ascertained before a water business is established. In other cases, the contaminations found are clearly those of manipulation. Before a person undertakes to operate a water business he should be prepared both in equipment and in operating knowledge to turn out a product free from contamination. This is demonstrated to be commercially possible, without burdensome restrictions, by the number of firms already marketing water free from contamination. It is equally evident in the ability of other firms to produce clean water after the need of doing so has been emphasized by court action.

[1] Identifications were made by Dr. Charles Thom, of the Bureau of Chemistry.

The results clearly show that bottled waters can be made to conform to the requirements of the United States Public Health Service for drinking water furnished upon trains; that is, that not more than one 10 cc sample out of five should show the presence of *B. coli*.

## TABULATED DATA.

TABLE I.—*Results of the bacteriological examination of water collected from spring No. 1.*

| Description of sample. | Colonies of organisms per cc developing after— | | | Smallest quantity in which were found— | | |
|---|---|---|---|---|---|---|
| | 2 days' incubation on nutrient agar at 37° C. | 4 days' incubation on nutrient gelatin at 20° C. | | *B. coli.* | | Molds. |
| | | Total count. | Liquefiers. | At time of collection. | 2 days after collection. | |
| | | | | cc. | cc. | cc. |
| "Clean" bottle rinsed with 100 cc sterile water | 1,000,000 | 1,400,000 | 17,000 | 0.1 | 0.01 | ..... |
| Do | 900,000 | 540,000 | 800 | .1 | 1.0 | ..... |
| "Dirty" bottle rinsed with 100 cc sterile water | 700,000 | 1,100,000 | 120,000 | .01 | .001 | ..... |
| Do | 1,000,000 | 1,400,000 | 59,000 | .01 | .001 | ..... |
| 16 caps rinsed with 70 cc sterile water | 4,800 | 700 | 18 | 1.0 | 5.0 | ..... |
| Water used for washing and rinsing bottles | 790,000 | 400,000 | 18,000 | .1 | .01 | ..... |
| Do | 840,000 | 1,000,000 | 90,000 | .1 | .1 | ..... |
| Water from bottling spring | 3,000 | 48,000 | 1,000 | [1] | [1] | 0.001 |
| Do | 4,500 | 38,000 | 1,000 | [1] | [1] | .001 |
| Water from creek 100 feet from bottling spring | 410,000 | 900,000 | 10,000 | .01 | .001 | ..... |
| Water from swimming pool, after use by 25 people | ..... | ..... | ..... | .01 | ..... | ..... |
| Water from swimming pool, after use by 170 people | ..... | ..... | ..... | .001 | ..... | ..... |
| 12 bottles collected after inspection; average results | 126,000 | 152,400 | 5,150 | [2].001 | ..... | .001 |

[1] No *B. coli* were present in 10 cc quantities.
[2] This determination was made at the time the sample was received at the laboratory.

TABLE II.—*Results of the bacteriological examination of water collected from spring No. 2.*

| Description of sample. | Colonies of organisms per cc developing after— | | | Smallest quantity in which were found— | | |
|---|---|---|---|---|---|---|
| | 2 days' incubation on nutrient agar at 37° C. | 4 days' incubation on nutrient gelatin at 20° C. | | *B. coli.* | | Molds. |
| | | Total count. | Liquefiers. | At time of collection. | 2 days after collection. | |
| | | | | cc. | cc. | cc. |
| "Clean" bottle rinsed with 100 cc sterile water | 280,000 | 800,000 | 800 | 0.1 | 1.0 | ..... |
| Do | 300,000 | 500,000 | 33,000 | 1.0 | 1.0 | 0.001 |
| 12 caps rinsed with 100 cc sterile water | 870 | 1,100 | 100 | 5.0 | 5.0 | ..... |
| Water from bottling spring | 137,000 | 110,000 | 2,000 | 1.0 | .1 | ..... |
| Do | 117,000 | 85,000 | 1,100 | 1.0 | .1 | ..... |
| Water from creek 5 feet from bottling spring | 310,000 | [1] | ..... | .001 | .0001 | ..... |
| Do | 297,000 | [1] | ..... | .001 | .0001 | ..... |
| 10 bottles collected after inspection; average results | 2,220 | 2,262 | 98 | [2].1 | ..... | ..... |

[1] Liquefied. [2] This determination was made at the time the sample was received at the laboratory.

TABLE III.—*Results of the bacteriological examination of water collected from spring No. 3.*

| Description of sample. | Colonies of organisms per cc developing after— | | | Smallest quantity in which were found— | | |
|---|---|---|---|---|---|---|
| | 2 days' incubation on nutrient agar at 37° C. | 4 days' incubation on nutrient gelatin at 20° C. | | *B. coli.* | | Molds. |
| | | Total count. | Liquefiers. | At time of collection. | 2 days after collection. | |
| | | | | cc. | cc. | cc. |
| "Clean" bottle rinsed with 100 cc sterile water | 2,700 | 3,700 | 110 | 1.0 | 1.0 | ..... |
| "Dirty" bottle rinsed with 100 cc sterile water | 37,000 | 40,000 | 3,300 | .01 | .01 | ..... |
| Water used for washing and rinsing bottles | 1,000 | 2,100 | 30 | .1 | .1 | ..... |
| Do | 1,700 | 1,500 | 40 | .1 | .1 | ..... |
| Water from bottling spring | 14 | 4 | 0 | [1] | [1] | ..... |
| Do | 8 | 3 | 0 | [1] | [1] | ..... |
| Water from receiving tank from spring | 330 | 290 | 190 | 10.0 | 10.0 | ..... |
| Do | 110 | 170 | 60 | 10.0 | 10.0 | ..... |
| Water from feeding tank for bottling | 170 | 3,100 | 0 | 1.0 | 5.0 | ..... |
| 6 bottles collected before inspection; average results | 10,100 | 33,500 | 313 | [2].01 | ..... | ..... |

[1] No *B. coli* were present in 10 cc quantities.
[2] This determination was made at the time the sample was received at the laboratory.

TABLE IV.—*Summary of the bacteriological examinations of water from American springs.*

| Spring No. | Number of samples. | Number of bottles. | Number of colonies of bacteria developing on nutrient agar or gelatin— | | | | | | Number of bottles showing *B. coli* in— | | | | | | Molds. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | At low temperature (20° or 25° C.). | | | At 37° C. | | | 10 cc. | 5 cc. | 1 cc. | 0.1 cc. | 0.01 cc. | 0.001 cc. | |
| | | | Highest. | Lowest. | Average. | Highest. | Lowest. | Average. | | | | | | | |
| 1 | 3 | 21 | 25,000 | 210 | 5,784 | 1,120 | 6 | 250 | 0 | 0 | 0 | 0 | 0 | 0 | Molds in 4 bottles. |
| 2 | 2 | 8 | 11,200 | 0 | 2,080 | 1,900 | 0 | 556 | 5 | 2 | 2 | 2 | 2 | 0 | |
| 3 | 4 | 36 | 8,600 | 300 | 2,410 | 6,600 | 41 | 1,200 | 30 | 23 | 19 | 7 | 0 | 0 | Molds in 2 bottles; Trichoderma, Penicillium, Cladosporium. |
| 4 | 1 | 6 | 5 | 0 | 2 | 2 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 5 | 1 | 12 | 10,200 | 29 | 1,782 | 11,100 | 29 | 1,700 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 6 | 1 | 6 | 4,500 | 400 | 1,483 | 1,000 | 160 | 643 | 4 | 3 | 2 | 0 | 0 | 0 | |
| 7 | 3 | 25 | 39,000 | 600 | 5,400 | 4,600 | 190 | 720 | 25 | 25 | 23 | 16 | 5 | 2 | |
| 8 | 2 | 6 | 6 | 3 | 5 | 3 | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 9 | 1 | 6 | 15,000 | 1,000 | 7,000 | 16,000 | 1,000 | 8,000 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 10 | 1 | 10 | 4,000 | 30 | 638 | 30 | 0 | 6 | 5 | 4 | 4 | 1 | 0 | 0 | |
| 11 | 1 | 9 | 5,700 | 700 | 2,300 | 1,700 | 40 | 440 | 3 | 3 | 0 | 0 | 0 | 0 | |
| 12 | 2 | 12 | 12,000 | 420 | 2,775 | 3,200 | 70 | 704 | 6 | 6 | 6 | 3 | 2 | 0 | |
| 13 | 4 | 15 | 11,500 | 700 | 4,250 | 8,000 | 700 | 3,837 | 10 | 10 | 10 | 8 | 3 | 0 | |
| 14 | 3 | 18 | 2,100 | 29 | 755 | 1,400 | 2 | 605 | 2 | 1 | 1 | 1 | 0 | 0 | Molds in 4 bottles. |
| 15 | 6 | 20 | 1,400 | 1 | 286 | 27,000 | 30 | 1,994 | 13 | 12 | 10 | 6 | 4 | 0 | |
| 16 | 4 | 33 | 1,480,000 | 270 | 132,050 | 360,000 | 610 | 34,982 | 15 | 12 | 9 | 8 | 5 | 0 | |
| 17 | 3 | 4 | 22,000 | 110 | 5,805 | 700 | 200 | 485 | 1 | 1 | 1 | 1 | 0 | 0 | |
| 18 | 1 | 6 | 120 | 20 | 55 | 900 | 90 | 381 | 1 | 0 | 0 | 0 | 0 | 0 | |
| 19 | 2 | 12 | 7,000 | 0 | 1,668 | 5,000 | 100 | 1,376 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 20 | 3 | 20 | 4,000 | 20 | 1,159 | 14,000 | 170 | 2,510 | 1 | 1 | 0 | 0 | 0 | 0 | |
| 21 | 4 | 21 | 3,100 | 0 | 821 | 28,600 | 22 | 4,680 | 5 | 5 | 5 | 4 | 3 | 0 | |
| 22 | 2 | 18 | 44,000 | 700 | 7,469 | 27,000 | 400 | 7,654 | 12 | 12 | 12 | 6 | 5 | 1 | |
| 23 | 1 | 11 | 125,000 | 200 | 15,165 | 94,000 | 20 | 8,973 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 24 | 1 | 1 | | | 8,000 | | | 2,000 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 25 | 2 | 12 | 1,340 | 81 | 453 | 24 | 0 | 8 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 26 | 2 | 24 | 500,000 | 200 | 12,430 | 70,000 | 1 | 14,384 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 27 | 3 | 18 | 10,100 | 440 | 2,875 | 5,000 | 38 | 666 | 6 | 4 | 2 | 1 | 0 | 0 | |
| 28 | 4 | 47 | 208,000 | 400 | 47,408 | 147,000 | 3,300 | 42,053 | 29 | 27 | 25 | 17 | 16 | 13 | |
| 29 | 1 | 11 | 12,000 | 0 | 1,128 | 8 | 0 | 3 | 3 | 0 | 0 | 0 | 0 | 0 | |
| 30 | 1 | 6 | 1,200 | 70 | 461 | 120 | 17 | 49 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 31 | 1 | 6 | 18,000 | 1,500 | 6,066 | 50,000 | 4,200 | 15,533 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 32 | 3 | 20 | 24,000 | 1,400 | 11,665 | 5,000 | 140 | 12,675 | 19 | 14 | 14 | 9 | 1 | 0 | |
| 33 | 4 | 25 | 126,000 | 90 | 6,712 | 50,000 | 40 | 3,258 | 14 | 12 | 0 | 0 | 0 | 0 | Molds in 4 bottles. |
| 34 | 5 | 24 | 22,000 | 90 | 3,625 | 33,000 | 2 | 2,566 | 9 | 8 | 4 | 1 | 0 | 0 | |

TABLE IV.—*Summary of the bacteriological examinations of water from American springs*—Continued.

| Spring No. | Number of samples. | Number of bottles. | Number of colonies of bacteria developing on nutrient agar or gelatin— | | | | | | Number of bottles showing *B. coli* in— | | | | | | Molds. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | At low temperature (20° or 25° C.). | | | At 37° C. | | | 10 cc. | 5 cc. | 1 cc. | 0.1 cc. | 0.01 cc. | 0.001 cc. | |
| | | | Highest. | Lowest. | Average. | Highest. | Lowest. | Average. | | | | | | | |
| 35 | 4 | 24 | 8,300 | 320 | 3,025 | 4,500 | 4 | 935 | 10 | 8 | 5 | 1 | 0 | 0 | |
| 36 | 3 | 7 | 2,800 | 70 | 1,015 | 260 | 90 | 166 | 3 | 3 | 3 | 2 | 2 | 0 | |
| 37 | 3 | 22 | 12,500 | 70 | 1,863 | 1,550 | 60 | 289 | 15 | 15 | 13 | 11 | 4 | 0 | |
| 38 | 1 | 6 | 23,000 | 4,700 | 12,000 | 8,200 | 900 | 3,700 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 39 | 1 | 6 | 6,400 | 90 | 1,948 | 800 | 90 | 276 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 40 | 2 | 6 | 5,200 | 50 | 238 | 3,000 | 70 | 671 | 4 | 4 | 4 | 3 | 2 | 0 | |
| 41 | 1 | 6 | 3,100 | 460 | 1,303 | 490 | 11 | 152 | 6 | 6 | 6 | 3 | 0 | 0 | |
| 42 | 1 | 12 | 400 | 4 | 107 | 1,129 | 61 | 248 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 43 | 1 | 5 | 400 | 80 | 252 | 200 | 70 | 127 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 44 | 2 | 12 | 16,200 | 2,000 | 9,175 | 9,900 | 1,000 | 4,816 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 45 | 3 | 24 | 81,000 | 4,000 | 39,140 | 32,000 | 1 | 12,867 | 12 | 12 | 12 | 10 | 2 | 0 | |
| 46 | 2 | 18 | 84,000 | 3,000 | 38,916 | 101,000 | 18,000 | 48,333 | 8 | 7 | 4 | 2 | 0 | 0 | |
| 47 | 2 | 11 | 3,250 | 60 | 894 | 2,000 | 1,000 | 1,500 | 11 | 11 | 11 | 11 | 0 | 0 | |
| 48 | 1 | 1 | ..... | ..... | 2,000 | ..... | ..... | 8,000 | 1 | 1 | 1 | 0 | 0 | 0 | |
| 49 | 1 | 6 | 7,000 | 2,000 | 3,616 | ..... | ..... | ..... | 4 | 4 | 4 | 0 | 0 | 0 | |
| 50 | 1 | 1 | ..... | ..... | 140 | ..... | ..... | ..... | 1 | 1 | 1 | 1 | 0 | 0 | |
| 51 | 1 | 6 | 33 | 3 | 10 | 7 | 1 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 52 | 1 | 12 | 7 | 0 | 3 | 54 | 2 | 10 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 53 | 8 | 69 | 66,000 | 0 | 8,642 | 16,400 | 30 | 490 | 48 | 47 | 36 | 15 | 5 | 0 | |
| 54 | 1 | 2 | 3,400 | 1,500 | 2,500 | 5,000 | 1,700 | 3,350 | 2 | 2 | 2 | 2 | 1 | 0 | |
| 55 | 2 | 18 | 170,000 | 3,300 | 50,000 | 135,000 | 700 | 40,000 | 18 | 17 | 15 | 14 | 11 | 10 | |
| 56 | 4 | 27 | 58,000 | 130 | 9,401 | 27,000 | 50 | 2,026 | 15 | 15 | 14 | 13 | 6 | 1 | |
| 57 | 1 | 11 | 4,800 | 80 | 828 | 550 | 55 | 220 | 7 | 5 | 1 | 0 | 0 | 0 | Penicillium and Citromyces in 11 bottles. |
| 58 | 1 | 12 | 70,400 | 820 | 13,593 | 42,700 | 60 | 6,479 | 5 | 3 | 3 | 2 | 1 | 0 | |
| 59 | 1 | 1 | ..... | ..... | 20,600 | ..... | ..... | ..... | 1 | 1 | 1 | 1 | 1 | 0 | |
| 60 | 1 | 2 | 19,000 | 14,000 | 16,500 | 16,000 | 11,000 | 13,500 | 2 | 2 | 2 | 2 | 1 | 1 | |
| 61 | 1 | 6 | 1,350 | 650 | 955 | ..... | ..... | ..... | 0 | 0 | 0 | 0 | 0 | 0 | |
| 62 | 1 | 3 | 1,800 | 500 | 9,233 | 3,500 | 600 | 1,606 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 63 | 1 | 6 | 79,000 | 7,200 | 41,533 | 3,400 | 1,100 | 1,266 | 6 | 6 | 6 | 6 | 6 | 1 | |
| 64 | 1 | 6 | 17 | 0 | 3 | 3 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 65 | 1 | 6 | 1,750 | 370 | 818 | ..... | ..... | ..... | ..... | ..... | 1 | 0 | 0 | 0 | |
| 66 | 1 | 6 | 65,000 | 6,000 | 17,050 | 2,900 | 90 | 976 | 6 | 6 | 6 | 0 | 0 | 0 | Molds in 0.001 cc dilution in every bottle. |
| 67 | 1 | 12 | 270 | 0 | 62 | 16 | 0 | 1+ | 0 | 0 | 0 | 0 | 0 | 0 | |
| 68 | 4 | 36 | 50,000 | 225 | 13,673 | 30,000 | 60 | 5,300 | ..... | ..... | 4 | 2 | 0 | 0 | |
| 69 | 4 | 19 | 14,000 | 10 | 2,468 | 6,000 | 10 | 1,134 | 5 | 4 | 4 | 0 | 0 | 0 | |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 70 | 2 | 9 | 37,000 | 120 | 7,967 | 3,000 | 10 | 516 | 0 | 0 | 0 | 0 | 0 | 0 | Sporotrichum in 9 bottles in 0.001 cc dilution. |
| 71 | 3 | 30 | 53,000 | 3 | 2,863 | 26,000 | 2 | 1,211 | 23 | 22 | 17 | 6 | 4 | 1 | |
| 72 | 1 | 3 | 80 | 12 | 55 | 330 | 80 | 173 | ---- | ---- | 0 | 0 | 0 | 0 | |
| 73 | 2 | 18 | 68,000 | 210 | 8,565 | 18,000 | 100 | 3,828 | 6 | 6 | 5 | 3 | 3 | 0 | |
| 74 | 2 | 18 | 1,500 | 12 | 269 | 14,000 | 1 | 894 | 6 | 5 | 5 | 3 | 0 | 0 | |
| 75 | 1 | 2 | 9,000 | 5,000 | 7,000 | 6,000 | 6,000 | 6,000 | 2 | 2 | 2 | 2 | 2 | 0 | |
| 76 | 1 | 10 | 24,000 | 200 | 2,170 | 22,000 | 120 | 5,882 | 10 | 10 | 10 | 10 | 10 | 8 | |
| 77 | 2 | 12 | 19,000 | 600 | 5,516 | 2,600 | 60 | 571 | 9 | 8 | 8 | 8 | 6 | 4 | |
| 78 | 2 | 5 | 26,000 | 500 | 2,266 | 2,200 | 100 | 1,226 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 79 | 3 | 36 | 92,000 | 300 | 12,680 | 82,000 | 50 | 5,890 | 33 | 28 | 23 | 6 | 3 | 0 | |
| 80 | 2 | 12 | 48,500 | 1,400 | 15,102 | 6,000 | 900 | 3,050 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 81 | 2 | 12 | 5,600 | 220 | 2,812 | 3,600 | 40 | 1,242 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 82 | 1 | 6 | 6,500 | 980 | 3,180 | ---- | ---- | ---- | 6 | 6 | 6 | 6 | 3 | 0 | |
| 83 | 1 | 6 | 160 | 60 | 98 | ---- | ---- | ---- | ---- | ---- | 2 | 0 | 0 | 0 | |
| 84 | 5 | 17 | 2,000 | 27 | 405 | 230 | 2 | 46 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 85 | 2 | 18 | 31,000 | 7 | 2,934 | 2,000 | 2 | 399 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 86 | 1 | 12 | 660,000 | 72,000 | 136,666 | 101,000 | 5,400 | 37,390 | 2 | 1 | 1 | 0 | 0 | 0 | |
| 87 | 1 | 6 | 57,000 | 200 | 13,613 | 83,000 | 13 | 1,577 | 5 | 5 | 1 | 0 | 0 | 0 | |
| 88 | 1 | 6 | 340,000 | 1,000 | 177,500 | 340,000 | 330 | 191,238 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 89 | 3 | 24 | 16,000 | 20 | 3,795 | 8,000 | 1 | 1,470 | 8 | 8 | 8 | 1 | 0 | 0 | |
| 90 | 1 | 1 | ---- | ---- | ---- | ---- | ---- | 70 | ---- | ---- | 0 | 0 | 0 | 0 | |
| 91 | 1 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 92 | 1 | 10 | 26,000 | 1,900 | 6,730 | 8,500 | 360 | 3,247 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 93 | 2 | 8 | 18,000 | 2,000 | 5,983 | 300,000 | 22 | 51,609 | 2 | 2 | 0 | 0 | 0 | 0 | |
| 94 | 1 | 12 | 1,040 | 0 | 139 | 810 | 0 | 69 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 95 | 2 | 8 | 73,000 | 40 | 1,008 | 102,000 | 64 | 13,721 | 8 | 8 | 6 | 3 | 0 | 0 | |
| 96 | 1 | 6 | 70,000 | 9,900 | 37,483 | 27,000 | 4,000 | 11,833 | 1 | 1 | 0 | 0 | 0 | 0 | |
| 97 | 1 | 6 | 1,200 | 110 | 656 | 200 | 40 | 115 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 98 | 4 | 32 | 10,000 | 3 | 540 | 590 | 0 | 78 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 99 | 1 | 6 | 65,200 | 3,600 | 12,450 | 36,000 | 1,100 | 8,983 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 100 | 4 | 16 | 49,000 | 200 | 6,750 | 27,000 | 40 | 5,440 | 9 | 7 | 7 | 3 | 1 | 0 | |
| 101 | 1 | 11 | 370 | 0 | 86 | 24 | 0 | 56 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 102 | 17 | 96 | 76,000 | 30 | 7,033 | 62,000 | 30 | 4,894 | 88 | 79 | 64 | 36 | 11 | 8 | Molds in 32 bottles. |
| 103 | 3 | 18 | 27,000 | 2 | 1,806 | 27,000 | 0 | 1,700 | 7 | 7 | 6 | 2 | 0 | 0 | |
| 104 | 1 | 6 | ---- | ---- | ---- | 32,000 | 5,200 | 6,280 | ---- | ---- | 0 | 0 | 0 | 0 | |
| 105 | 1 | 6 | 1,300 | 220 | 2,061 | 160 | 6 | 62 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 106 | 1 | 12 | 5,500 | 60 | ---- | 14,000 | 50 | 2,150 | 9 | 9 | 9 | 2 | 1 | 0 | |
| 107 | 3 | 5 | 2,500 | 170 | 1,118 | 1,200 | 33 | 400 | 4 | 4 | 3 | 2 | 0 | 0 | |
| 108 | 1 | 12 | 12,000 | 140 | 2,410 | 5,000 | 10 | 600 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 109 | 3 | 18 | 700 | 0 | 223 | 600 | 2 | 200 | 12 | 11 | 11 | 10 | 6 | 1 | |
| 110 | 2 | 11 | 7,300 | 1,600 | 4,300 | 5,400 | 90 | 1,246 | 11 | 11 | 9 | 4 | 0 | 0 | |

TABLE V.—*Summary of the bacteriological examinations of samples of imported waters*

| Spring No. | Number of samples. | Number of bottles. | Number of colonies of bacteria developing on nutrient agar or gelatin— | | | | | | Number of bottles showing *B. coli* in— | | | | | | Molds. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | At low temperature (20° or 25° C.). | | | At 37° C. | | | 10 cc. | 5 cc. | 1 cc. | 0.1 cc. | 0.001 cc. | 0.001 cc. | |
| | | | Highest. | Lowest. | Average. | Highest. | Lowest. | Average. | | | | | | | |
| 1 | 1 | 3 | 70 | 12 | 40 | 80 | 7 | 40 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 2 | 2 | 5 | 25,000 | 440 | 6,148 | 14,000 | 30 | 2,832 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 3 | 1 | 5 | 31 | 2 | 11 | 18 | 0 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 4 | 5 | 36 | 800 | 0 | 58 | 250 | 0 | 18 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 5 | 1 | 6 | 1,200 | 0 | 390 | 540 | 1 | 334 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 6 | 1 | 5 | 3,900 | 450 | 1,612 | 190 | 5 | 78 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 7 | 1 | 1 | 5,000 | 5,000 | 5,000 | 3,000 | 3,000 | 3,000 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 8 | 1 | 4 | 30 | 0 | 10 | 120 | 2 | 32 | 2 | 2 | 2 | 2 | 0 | 0 | |
| 9 | 1 | 4 | 1 | 0 | | 4 | 3 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 10 | 2 | 5 | 210,000 | 3,300 | 130,000 | 840 | 110 | 424 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 11 | 1 | 4 | 1,100 | 140 | 567 | 900 | 400 | 622 | 4 | 4 | 4 | 2 | 2 | 0 | |
| 12 | 2 | 7 | 38 | 2 | 14 | 16 | 2 | 9 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 13 | 2 | 6 | 1,010 | 20 | 308 | 640 | 6 | 195 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 14 | 1 | 11 | 22,400 | 1,100 | 6,563 | 11 | 0 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 15 | 1 | 6 | 4,300 | 700 | 2,450 | 20,000 | 300 | 1,250 | 4 | 4 | 4 | 2 | 0 | 0 | |
| 16 | 1 | 2 | 20 | 0 | 10 | 800 | 110 | 455 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 17 | 2 | 6 | 10 | 1 | 4 | 560 | 50 | 225 | 2 | 1 | 0 | 0 | 0 | 0 | |
| 18 | 1 | 4 | 1,600 | 80 | 670 | 9,000 | 40 | 2,510 | 0 | 0 | 0 | 0 | 0 | 0 | Molds in 2 bottles. |
| 19 | 1 | 1 | | | 120 | | | 70 | 1 | 0 | 0 | 0 | 0 | 0 | |
| 20 | 1 | 4 | 1,400 | 40 | 652 | 3 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 21 | 1 | 5 | 21,000 | 1,500 | 7,400 | 4,000 | 500 | 1,220 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 22 | 1 | 1 | | | 35 | | | 20 | 1 | 0 | 0 | 0 | 0 | 0 | |
| 23 | 1 | 4 | 7,000 | 160 | 2,592 | 29,000 | 470 | 8,140 | 3 | 1 | 1 | 0 | 0 | 0 | |
| 24 | 1 | 4 | | | 0 | | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 25 | 6 | 39 | 3,000 | 3 | 255 | 400 | 0 | 82 | 20 | 19 | 14 | 4 | 0 | 0 | |
| 26 | 1 | 2 | 800 | 500 | 650 | 40 | 30 | 35 | 0 | 0 | 0 | 0 | 0 | 0 | Molds in 2 bottles. |
| 27 | 3 | 7 | 24,000 | 120 | 9,410 | 1,200 | 40 | 482 | 6 | 5 | 5 | 5 | 3 | 0 | |
| 28 | 1 | 6 | 8,200 | 72 | 2,745 | 70 | 0 | 16 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 29 | 5 | 14 | 11,000 | 40 | 1,405 | 6,000 | 0 | 960 | 7 | 7 | 4 | 4 | 2 | 0 | |
| 30 | 1 | 2 | 260 | 63 | 161 | 82 | 6 | 44 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 31 | 1 | 5 | 150 | 0 | 30 | 3 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 32 | 1 | 1 | | | 22 | | | 25 | 1 | 1 | 1 | 0 | 0 | 0 | |
| 33 | 1 | 6 | 10 | 0 | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 34 | 4 | 8 | 27,000 | 2 | 6,546 | 7,000 | 0 | 1,761 | 2 | 2 | 2 | 1 | 0 | 0 | |
| 35 | 2 | 6 | 120,000 | 4 | 44,500 | 8 | 1 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | |

| | | |

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